



Phylogenetic position of *Helicotylenchus multicinctus* on banana recorded in the municipality of Limoeiro do Norte, Brazil

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ABSTRACT

The spiral nematode, *Helicotylenchus multicinctus*, is present in production areas of various agricultural crops around the world and in Brazil, standing out as the main nematode causing damage to banana cultivation. Given the importance of this pathogen, this work aimed to determine the sequence of the ITS and 28S rDNA regions of *H. multicinctus* and explore the phylogenetic position of *H. multicinctus* isolated from Brazil. After collection, nematodes were extracted from the samples and morphological and molecular identification was carried out and, through Bayesian analysis of 28S rDNA, the phylogenetic position of *H. multicinctus* was inferred. The morphological and molecular data of this study identified *H. multicinctus* associated with banana farming in Limoeiro do Norte and *H. multicinctus* from Brazil grouped with the other *H. multicinctus* isolates in the world presenting high Bootstrap values within Clade XI.

KEYWORDS: Spiral nematode. *Musa* spp. Molecular. 28S.

Posição filogenética de *Helicotylenchus multicinctus* em banana registrado no município de Limoeiro do Norte, Brasil

RESUMO

O nematoide espiralado, *Helicotylenchus multicinctus*, está presente em áreas de produção de diversas culturas agrícolas no mundo e no Brasil, destacando-se como o principal fitonematoide causador de prejuízos no cultivo da banana. Diante da importância deste patógeno, este trabalho teve como objetivo determinar a sequência das regiões ITS e 28S rDNA de *H. multicinctus* e explorar a posição filogenética de *H. multicinctus* isolados do Brasil. Após as coletas, os nematoides foram extraídos das amostras e procedeu-se a identificação morfológica e molecular e através da análise bayesiana do 28S rDNA foi feita a inferência da posição filogenética de *H. multicinctus*. Os dados morfológicos e moleculares deste estudo identificaram *H. multicinctus* associado à bananicultura em Limoeiro do Norte e *H. multicinctus* do Brasil agrupado com os demais isolados de *H. multicinctus* do mundo apresentando altos valores de Bootstrap dentro do Clado XI.

PALAVRAS-CHAVE: Nematoide espiralado. *Musa* spp. Molecular. 28S.

1 INTRODUÇÃO

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The banana (*Musa* spp.) is included as a basic food in the human diet due to its energy, mineral, and vitamin value, being among the main crops in the tropics and subtropics (Robinson; Galán Saúco, 2010) and being the most produced fruit in the world (FAO, 2021). In Brazil, banana cultivation has 456,375 ha planted, with 6,811,374 t produced, with emphasis on the Northeast region, which is responsible for approximately 18% of national production, and the state of Ceará which is among the main producers (IBGE, 2021).

Nematodes, particularly *Radopholus similis* (Cobb, 1893a) Thorne 1949 and *Helicotylenchus multicinctus* (Cobb, 1893b) Golden, 1956, alongside *Meloidogyne* spp. and *Pratylenchus* spp., are key contributors to banana production area damage. In Brazil, nematode surveys carried out in banana crops have shown a high incidence of *H. multicinctus* (Luquini *et al.*, 2019), emphasizing its importance as a causal agent of damage in this culture. The genus *Helicotylenchus* Steiner represents the "spiral nematodes", those whose shape becomes completely spiral when dead, but which when alive are threadlike. The *Helicotylenchus* specimens have a wide range of hosts and are present in different regions of the world, with more than 200 recognized species, with *H. multicinctus* and *H. dihystra* (Cobb, 1893b) Sher, 1961 recognized as the main ones for causing economic losses in agricultural crops (Uzma *et al.*, 2015; Ferraz; Brown, 2016).

Morphological identification of *Helicotylenchus* species is unreliable due to interspecific variability, necessitating molecular approaches like nuclear rDNA sequencing. This method reveals phylogenetic differences among morphologically similar isolates, enhancing accuracy in identification (Gutiérrez-Gutiérrez *et al.*, 2010; Cantalapiedra-Navarrete *et al.*, 2013). Only 20% of *Helicotylenchus* species were sequenced with 28S rDNA, revealing 12 clades (I to XII) initially grouped by Subbotin *et al.* (2015) and later expanded by Rybarczyk-Mydlowska *et al.* (2019). For this classification, six isolates of *H. multicinctus* belonging to Africa and North America were analyzed, therefore, without any isolate from South America.

In view of the above, the objectives of this study were to provide molecular characteristics of *H. multicinctus* occurring in Ceará state, Brazil, through the use of ITS and 28S rDNA data, and to determine the phylogenetic position of *H. multicinctus*, isolated from Brazil, with other *Helicotylenchus* species using the sequences 28S rDNA D2-D3. In October 2019, soil and root samples were collected from a banana tree in the municipality of Limoeiro do Norte, Ceará state, Brazil (5°10'57"S, 38°00'37"W). Nematodes were extracted from roots and soil according to Coolen and D'Herde (1972) and Jenkins (1964), respectively. Morphological identification (n = 20) was performed using the identification key and descriptions by Uzma *et al.* (2015).

Genomic DNA from single nematode specimens was extracted either GenElute™ Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich) or QIAamp DNA Micro Kit (Qiagen) according to the manufacturers instructions, and the quality and quantity of DNA were estimated, respectively, in 0.8% agarose gel and NanoVue™ (GE Healthcare ®). Molecular identification of *Helicotylenchus* specimens was performed by amplification and sequencing of the ITS region VRAIN2F-VRAIN2R (forward 5'-CTTTGTACACACCGCCGTCGCT-3' and reverse 5'-TTTCACTCGCCGTTACTAAGGGAATC-3') and the 28S gene D2-D3 fragment (forward 5'-ACAAGTACCGTGAGGGAAAGTTG-3' and reverse 5'-TCG-GAAGGAACCAGCTACTA-3') (Vrain *et al.*, 1992; De Ley *et al.*, 1999). PCR cycle conditions for the ribosomal DNA markers were described by Maafi *et al.* (2003). All consensus sequences obtained were used to compare against the NCBI nucleotide database, based on BLASTn search. The phylogenetic analysis utilized the maximum likelihood (ML) executed through RAXML-HCP2 v.8.2.8 (Stamatakis, 2014), which was implemented on CIPRES Portal v.2.0 (<https://www.phylo.org/portal2/home.action>) with 1,000 Bootstrap repetitions. The ML analysis employed a GTR-GAMMA model of evolution. Only one haplotype from each sequenced region was used.

The morphological and molecular data of this study identified *H. multicinctus* CN0022 extracted from banana plantations in Brazil (Figure 1). The females of *H. multicinctus* CN0022 showed the stylet measured 24.1 µm; vulva position was 64.5% of body length; and tail measurements were c (relationship

between body length and tail length) = 50.1 μm , **c'** (relationship between tail length and tail diameter at anus height) = 1.0 μm and DGO (distance from stylet nodules to dorsal gland opening) = 8.8 μm . Total body length was 596.1 μm , with **a** (relationship between body length and tail length) = 29.1 μm , **b** (relationship between body length and distance from anterior end to pharyngo-intestinal junction) = 5.42 μm , and **b'** (relationship between body length and distance from anterior end to esophagus end) = 4.75 μm . These data were consistent with the original description of this species (Siddiqi, 1973).

Figure 1: *Helicotylenchus multicinctus* female. Visualization of the vulva (black arrow) and anterior region (white arrow).

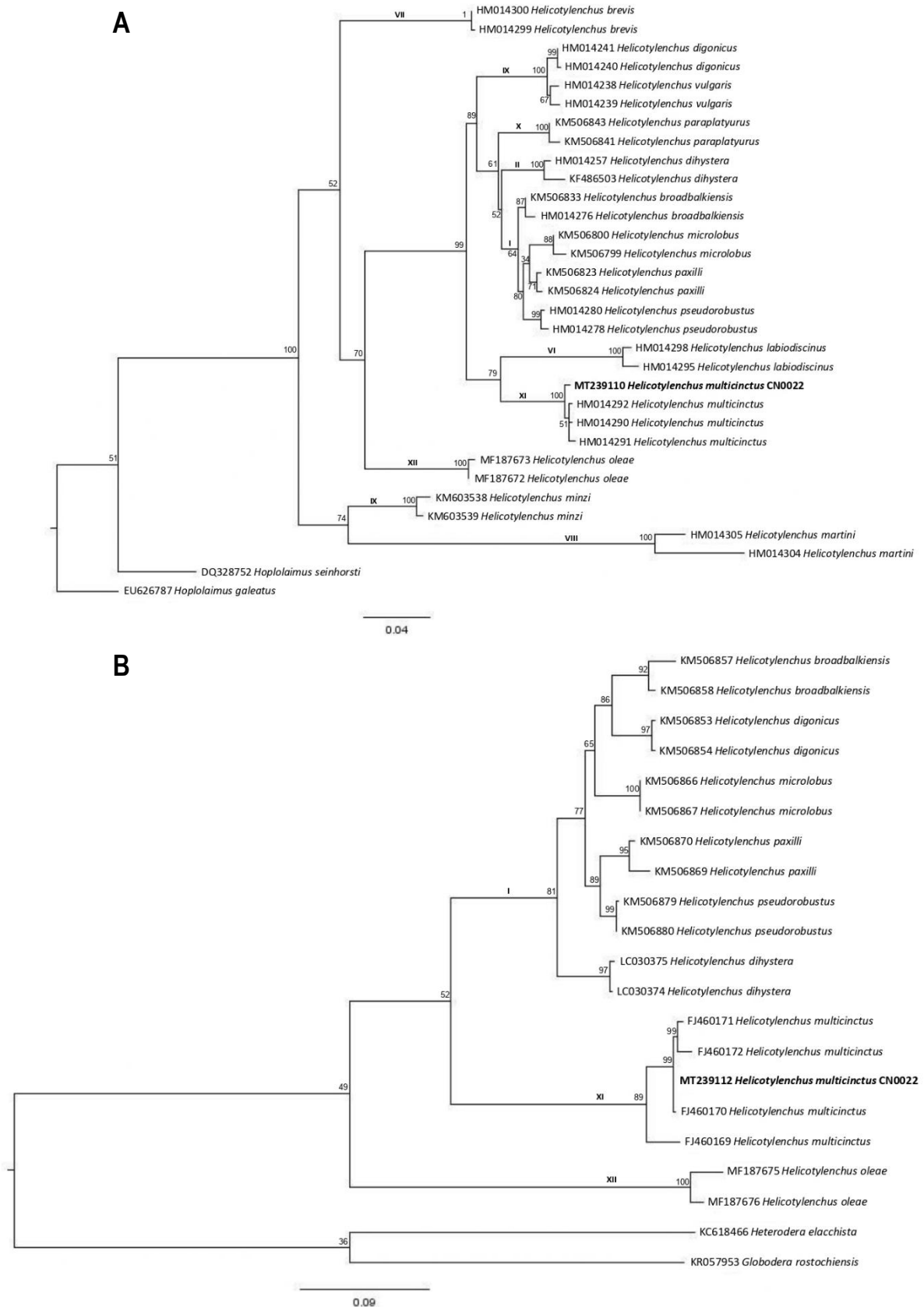


Source: Research Data, 2023.

They were obtained amplicons of 792 and 1,214 bp were obtained and sequenced for D2–D3 of the 28S and ITS rDNA regions, respectively. The sequences of the studied rDNA regions were submitted to GenBank. The *H. multicinctus* CN0022 populations used for molecular analysis showed for sequences alignments for ITS region a high degree of sequence identity (99%) with *H. multicinctus* from Malaysia and China (FJ460173 and KF443216) differing by 4 to 10 bp and zero indels, respectively, according to a BLASTn search. The identity of the D2/D3 sequence of *H. multicinctus* CN0022 was 99% with *H. multicinctus* isolates from China and South Africa (KF443214 and MN056344) differing by 1 bp and zero indels for both.

Phylogenetic analysis using BI of ITS rDNA sequences derived from *Helicotylenchus* spp. are provided in Figure 2A, in agreement with the phylogenetic data from Subbotin *et al.* (2015). Eight highly supported clades can be distinguished, including *Heterodera elachista* Ohshima, 1974 and *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 that were selected as an outgroup. The IB analysis of 28S rDNA of *Helicotylenchus* species produced a phylogenetic tree shown in Figure 2B. The phylogenetic

Figure 2: Phylogenetic relationship of *Helicotylenchus* species based on sequence alignment of the ITS rDNA region (A), *Heterodera elachista* and *Globodera rostochiensis* were used as outgroups, and 28S rDNA D2-D3 expansion fragments (B), *Hoplolaimus seinhorsti* and *Hoplolaimus galeatus* were used as



Source: Research Data, 2023.

tree was constructed based on 32 taxa including *Hoplolaimus seinhorsti* Luc, 1958 and *H. galeatus* (Cobb, 1913) Thorne, 1935 as the outgroup. The *H. multicinctus* CN0022 isolated from banana grouped with *H. multicinctus* with high Bootstrap values, within Clade XI according to Rybarczyk-Mydlowska *et al.* (2019) and Subbotin *et al.* (2015). The morphological evaluation of *H. multicinctus* nematodes and their position in Clade XI of the *Helicotylenchus* 28S rDNA tree agreed with the characterization of the species of this species, as described in Subbotin *et al.* (2015) and Rybarczyk-Mydlowska *et al.* (2019).

With the availability of the sequences from the 28S and ITS regions rDNA provided in this study, the molecular characteristics of *H. multicinctus* were increased, mainly with the addition of molecular data from the world's fourth largest banana producing country. This is the first report of the isolation and molecular characterization of *H. multicinctus* in *Musa* spp. in the municipality of Limoeiro do Norte, Ceará state.

CONTRIBUIÇÃO DOS AUTORES. FJCSJ: Conceituação, Metodologia, Software, Curadoria de dados, Análise formal; FJCSJ, MCA: Redação – rascunho original, Visualização, Investigação, Validação; FJCSJ, MCA: Redação – revisão e edição, Administração do projeto, Recursos, Supervisão. Todos os autores participaram ativamente da discussão dos resultados, revisaram e aprovaram a versão final do artigo.

CONFLITO DE INTERESSE. Os autores declaram que não têm interesse comercial ou associativo que represente um conflito de interesses em relação ao manuscrito.

APROVAÇÃO ÉTICA. Não se aplica.

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